

|                          |   |                         |                |
|--------------------------|---|-------------------------|----------------|
| <b>Department:</b>       | Laboratory and Blood Bank (Haematology) |                         |                |
| <b>Document:</b>         | Internal Policy and Procedure           |                         |                |
| <b>Title:</b>            | Colorimetric Assay of Protein C         |                         |                |
| <b>Applies To:</b>       | All Laboratory Staff                    |                         |                |
| <b>Preparation Date:</b> | January 07, 2025                        | <b>Index No:</b>        | LB-IPP-069     |
| <b>Approval Date:</b>    | January 21, 2025                        | <b>Version :</b>        | 2              |
| <b>Effective Date:</b>   | February 21, 2025                       | <b>Replacement No.:</b> | LB-IPP-069 (1) |
| <b>Review Date:</b>      | February 21, 2028                       | <b>No. of Pages:</b>    | 04             |

## 1. PURPOSE:

1.1 This describes the procedure for the quantitative determination of the functional protein C level in plasma by the synthetic chromogenic substrate method.

## 2. DEFINITONS:

2.1 Protein C is a vitamin -K dependent coagulation inhibitor that regulates the activity of Factors V and VIII. Protein-C, in inhibiting activated Factors V and VIII helps to limit thrombus extension, and thus, appears to be a major regulator of Coagulation process.

## 3. POLICY:

3.1 Protein C is activated by the specific activator (reagent 1) derived from the venom of Agkistrodon c. contortrix (The quantity of enzyme thus formed is measured by its amidasic activity on the synthetic chromogenic substrate CBS 42.46 (reagent 2). (Paranitroaniline release at 405 nm). The intensity of the color produced is directly proportional to the level of protein C initially present in the test plasma

## 4. PROCEDURE:

4.1 Specimen

- 4.1.1 Collect the blood in a blue stopper vacutainer tube using a ratio of 9 parts of whole blood and 1 part of 3.2% buffered sodium citrate.
- 4.1.2 Centrifuge the specimen at 3500 rpm for 10 minutes
- 4.1.3 Remove the platelet poor plasma and transfer to a 12 x 75 glass test tube. For the best results, test must be performed immediately.
- 4.1.4 Freeze the plasma at -20°C or lower until ready to test (MAX 2 weeks).
- 4.1.5 Frozen plasma must be thawed directly at 37°C for 15 minutes before testing.
- 4.1.6 Specimens are stable for 4 hours at +20°C and 2 weeks at -20°C.
- 4.1.7 Samples that have an abnormally high haematocrit, i.e. >55%, must be re-drawn into specially modified tubes that have had the volume of anticoagulant adjusted to ensure a correct ratio of blood to anticoagulant.

4.2 Procedural steps

- 4.2.1 Refer to STA- Compact Operation and maintenance procedure to help you in performing the assay.
- 4.2.2 Quality Control: performed with each run
- 4.2.3 System Control N.
- 4.2.4 System Control P.
- 4.2.5 Check values to ensure that results are within acceptable limits.
- 4.2.6 Patient results cannot be released if the run is rejected based on Westguard Rules.
- 4.2.7 Check reagents and controls for expiration date.

4.3 Expected values:  
4.3.1 70-130 % of the normal pooled plasma.  
4.3.2 Clinical Significance: Screening for thrombophilia in which protein C deficiency might be the cause

4.4 Limitation:  
4.4.1 On STA, protein C is insensitive to Hgb (up to 5 g/l), bilirubin (up to 200mg/l) and triglyceride (up to 10g/l)  
4.4.2 The presence of a protein in the plasma to be tested results in an under-estimation of protein C level

## 5. MATERIALS AND EQUIPMENT:

5.1 STA® - Owren-Koller (REF 00360).  
5.2 STA® - CaCl 0.025 M (REF 00367)  
5.3 STA® - Unicalibrator (REF 00675).  
5.4 System Control N+ P (REF 00678): kit containing control plasmas, normal and abnormal levels.  
5.5 Common clinical laboratory equipment and materials (centrifuge, distilled water...).  
5.6 Reagent 1: Reconstitute each vial with 3 ml of distilled water. Allow the reconstituted reagent to remain at room temperature (18°-25°C), for 60 min. Swirl vial gently; then, install an STA- mini reducer in the vial and the perforated cap on top. Reconstituted Stability in original vial with STA- mini reducer and perforated cap in place is 21 days on STA- Compact.  
5.7 Reagent 2: Reconstitute each vial with 6 ml of distilled water DON'T SHAKE. Allow the reconstituted reagent to remain at room temperature (18°-25°C), for 60 min, Swirl vial gently; then, install an STA- mini reducer in the vial. Reconstituted Stability in original vial with STAmi reducer and perforated cap in place is 21 days on STACcompact  
5.8 Each mini reducer is used only ONCE. Don't re-use it again.

## 6. RESPONSIBILITIES:

6.1 This policy applies to all Hematology technologists involved in this special Hematology test.

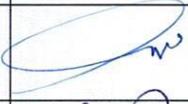
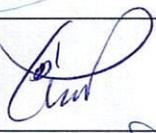
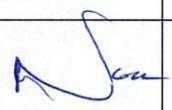
## 7. APPENDICES:

7.1 Reagent Preparation And Storage

## 8. REFERENCES:

8.1 Protein C inserts Kit.

9. APPROVALS:

|                     | Name                          | Title                              | Signature   | Date             |
|---------------------|-------------------------------|------------------------------------|---|------------------|
| <b>Prepared by:</b> | Dr. Fatma Hassan Ahmed        | Clinical Pathologist               |  | January 07, 2025 |
| <b>Reviewed by:</b> | Dr. Kawther M. Abdou          | Consultant & Lab. Medical Director |  | January 08, 2025 |
| <b>Reviewed by:</b> | Ms. Noora Melfi Alanizi       | Laboratory & Blood Bank Director   |  | January 09, 2025 |
| <b>Reviewed by:</b> | Mr. Abdulelah Ayed Al Mutairi | QM&PS Director                     |  | January 12, 2025 |
| <b>Reviewed by:</b> | Dr. Tamer Mohamed Naguib      | Medical Director                   |   | January 12, 2025 |
| <b>Approved by:</b> | Mr. Fahad Hazam Alshammari    | Hospital Director                  |  | January 21, 2025 |

## Appendix 7.1

### REAGENT PREPARATION AND STORAGE

| Reagents   | Preparation  | Stability after reconstitution/<br>opening on board STA Compact <sup>®</sup> | Storage position on STA Compact <sup>®</sup> |
|--|--|--|--|
| <b>Reagent 1<br/>(Protein S Deficient Plasma)</b>                                | Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Then, gently homogenize. | 4 hours  | Product drawer                               |
| <b>Reagent 2<br/>(PCa)</b>   | Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Then, gently homogenize. | 4 hours  | Product drawer                               |
| <b>Reagent 3<br/>(F. Va)</b>   | Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 60 minutes. Then, gently homogenize. | 4 hours  | Product drawer                               |
| <b>STA<sup>®</sup> - Owren-Koller</b>  | 15-ml vial. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes before use.  | 3 days   | Sample drawer                                |
| <b>STA<sup>®</sup> - CaCl<sub>2</sub> 0.025 M</b>                                | 15-ml vial. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes before use.  | 3 days   | Product drawer                               |
| <b>STA<sup>®</sup> - Unicalibrator</b>   | Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes. Then, homogenize.        | 4 hours  | Product drawer                               |
| <b>STA<sup>®</sup> - System Control N<br/>STA<sup>®</sup> - System Control P</b> | Add exactly 1 ml of distilled water. Allow the solution to stand at room temperature (18-25 °C) for 30 minutes. Then, homogenize.        | 8 hours  | Product drawer                               |